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GROWTH AND DEVELOPMENT OF CHRYSAORA QUINQUECIRRHA REARED UNDER DIFFERENT DIET COMPOSITIONS

Mestrado em Ecologia Marinha

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GROWTH AND DEVELOPMENT OF *CHRYSAORA QUINQUECIRRHA* UNDER 3 SEPARATE DIETS

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II. ABSTRACT

As a contribution to further understanding the growth and rearing in aquaculture of the jellyfish *Chrysaora quinquecirrha*, laboratorial experiments were conducted in order to evaluate key differences in growth in regards to different food items given to ephyras, identifying the best diet for this species and development stage, and thus contributing to the optimization of its aquaculture. 9 tanks were set up where medusa were fed 3 different diets in 3 replicate tanks each – 3 replicate tanks were fed enriched *Artemia nauplii*, 3 were fed *Acartia grani* copepods enriched with *Rhodomonas* sp. and the last 3 were fed minced *Aurelia aurita*. The main aim was to identify the diet that translated into maximum ephyra growth of *Chrysaora quinquecirrha* for a 31 day period (with 10 ephyras per tank with identical size and age). Ephyra pulses were monitored daily and there were no significant differences between ephyra grown with different diets, revealing that all prey elicited a similar feeding response. However, growth rates were significantly different for ephyra grown with different diets. The diet that resulted in highest growth rates of the ephyras consisted in minced *Aurelia aurita* (2.6mm ±SD over the course of 31 days), followed by the diet consisting of copepods (1.8mm ±SD over the course of 31 days) and finally the diet based on *Artemia nauplii* (1.08mm ±SD over the course of 31 days). This study recommends that the dietary supplementation with copepods or minced *Aurelia aurita* is an important enhancement of the management of *Chrysaora quinquecirrha ephyra* culture, and that growth rates can be elevated from 83.1% to 128.6% (*Acartia grani*) and 185.7% (*Aurelia aurita*) when compared to the traditional diet.

Keywords: Ephyras, *Chrysaora quinquecirrha*, Aquaculture rearing, Diet, Growth rates, Replicates.

Resumo: Como contributo para a compreensão do crescimento e criação em aquacultura da medusa *Chrysaora quinquecirrha*, experiências laboratoriais foram realizados a fim de avaliar as principais diferenças no crescimento em relação a diferentes itens alimentares fornecidos a éfiras, identificando a melhor dieta para esta fase e desenvolvimento da espécie e, assim, contribuindo para a optimização da sua aquacultura. 9 Tanques foram montados onde éfiras foram alimentadas com 3 dietas diferentes em cada 3 replicados – 3 replicados foram alimentadas com náuplios de *Artémia* enriquecida, 3 foram alimentados com copépodes *Acartia grani* enriquecidos com *Rhodomonas* sp. e os últimos e foram alimentadas com *Aurélia aurita* triturada. O principal objetivo foi identificar a dieta que se traduziu em crescimento máximo de éfiras de *Chrysaora quinquecirrha* por um período de 31 dias (com 10 éfiras por tanque com tamanho e idade idênticos) pulsações de éfiras foram monitorizadas diariamente e não houve diferenças significativas entre éfiras cultivadas com diferentes dietas, revelando que todas as presas revelaram uma resposta de alimentação semelhante. No entanto, as taxas de crescimento foram significativamente diferentes para éfiras alimentadas com as diferentes dietas. A dieta que resultou em maiores taxas de crescimento das éfiras consistiu em *Aurélia aurita* triturada (± DP 2,6 milímetros ao longo de 31 dias), seguido pela dieta de copépodes (± DP 1,8 milímetros ao longo de 31 dias) e finalmente a dieta baseada em náuplios de *Artémia* (± DP 1,08 milímetros ao longo de 31 dias). Este estudo recomenda que o suplemento dietético com copépodes ou *Aurélia aurita* triturada é um melhoramento importante na gestão da cultura de éfiras de *Chrysaora quinquecirrha*, e que as taxas de crescimento podem ser aumentadas de 83,1% a 128,6% (*Acartia grani*) e de 185,7% (*Aurélia aurita*) quando em comparação com a dieta tradicional.

Palavras-chave: Éfiras, *Chrysaora quinquecirrha*, Aquacultura, Dieta, taxas de crescimento, Replicados.

III. <u>INTRODUCTION</u>

In natural environments food sustenance and growth are critically related and impaired by natural occurring conditions, under the restrain of abiotic and biotic factors different species and individuals grow, reproduce and die.

Various species of medusae are known to greatly influence local food chains and nutrient flow (Widmer, 2006). In many open water systems, local temperature and food available will determine greatly intra and inter specific competition of medusae (usually these factors carry the most influence on a given population) (William K Fitt, 1997).

Jellyfish are known to easily proliferate in any given natural water system - even perturbed systems seem to ecologically benefit them over many other existing aquatic fauna. Certain life-history features help them thrive in disturbed systems (Hays, 2011), these are:

- Short generation times, (usually a couple of months in most species) meaning mass recruitment is done yearly (Collins, 2002).
- Highly reproductive yield, slow ephyra releasing polyps can generate vast amounts of offspring in many jellyfish species (Collins, 2002).
- Lack of natural predators - most commonly medusae species are predated by other medusae such is the case of *Chrysaora quinquecirrha* preying on *Aurelia aurita* (Purcell, 1992), although sea turtles and Sun fish are also recognized as main predators.

- Tactile predation - jellyfish are tactile predators,meaning that instead of sight it uses touch as a tool for foraging, as such they can be effective predators both during day and night and in the presence of waters with high turbidity (Gibbons, 2010), (Hays, 2011).

- Diversity of prey - they are large consumers of a huge diversity of zooplankton organisms and also of larvae and eggs and fish (some also prey on medusae) (Gibbons, 2010).

The medusae under study in this master thesis was the jellyfish *Chrysaora quinquecirrha,* commonly named "Atlantic sea nettle" as it is mostly found on the eastern coast of United States of.America, with particular reference to Chesapeake bay it has various other medusae species as neighbouring residents, although *Chrysaora quiquecirrha* can also be found in some areas of the Indo-Pacific. These medusae usually, appear in mass numbers from May up to the end of September but many sightings still occur all the way to November (Purcell, 1992).

The body of *Chrysaora quinquecirrha* is usually an outer epidermis cup/bell shape, inner gastrodermis layer and tentacles. The tentacles are quite elongated and thin growing up to several centimetres (more than 30). This dome shaped jellyfish commonly has 8 internal lobes for the growth of tentacles, aligned with nematocysts (organelles designed for stinging prey and predators). The Atlantic *Chrysaora* is usually white opaque in colour with small red streaks in its dome extremities.

Figure 1 – Adult *Chrysaora quinquecirrha*

III. 1. The medical potential of venom.

All venom is multifaceted and multitasking, many venom toxins target the same molecules that need to be controlled to treat diseases, in its essence it is a series of highly active proteins and peptides with an exceedingly functional enzymatic and toxic effect/action. (Holland, 2013)

Jellyfish venom is still in its very early extraction and analysis stages (Burnett, 1998), (Pane, 2013). As such, novel compounds can still be found and analysed today in the pharmacological department. (Pane, 2013)

In late 2006 some compounds were isolated that are believed to be beneficial in the treatment of heart disease. (Sydney Morning Herald, 2006). Even extraction and proper handling techniques are still very rudimentary in some cases and species, different removal methods yield different crude venom samples and protein samples are likely to be altered by this (Wiltshire, 2000) and not just individual specific lifestyles (Pane, 2013).

III. 2. Natural ecology and life cycle

Most scyphozoan jellyfishes have a two part life cycle: free-swimming medusa and bottom-dwelling polyp (although there are notable exceptions). The most noteworthy of these exceptions are the stauromedusae, these cnidarians are sessile in nature and will attach themselves to a surface during their larval/ephyra stage where they will remain and grow.

The free-swimming medusa (the part we call "a jellyfish") is either female or male and produces eggs or sperm released into the open water which combine to produce a larva, called a 'planula' (plural = planulae). The planula swims through the water to find a suitable place to settle, i.e. attach itself to a surface. In the marine lakes, *Mastigias* planulae settle on the surface (typically the sides or underneath) of rocks, rotting logs, and decaying leaves that accumulate around the lake's side in the poorly oxygenated (but not anoxic) waters at intermediate depths.

The planula metamorphoses into a sessile (i.e. fixed-position), usually benthic (i.e. bottom dwelling) polyp called a 'scyphistoma' and it is the scyphistoma, still attached to the surface on which the planula settled, that produces a new free-swimming medusa. The process by which new medusae are produced is called 'strobilation' and involves metamorphosis of the end of a scyphistoma into an 'ephyra', an immature medusa, which subsequently detaches and swims away.

Depending on the species, a single polyp may produce one or many ephyrae all at once, over a period of time, or at different intervals. The ephyra subsequently develops into a mature medusa over a period of weeks to months.

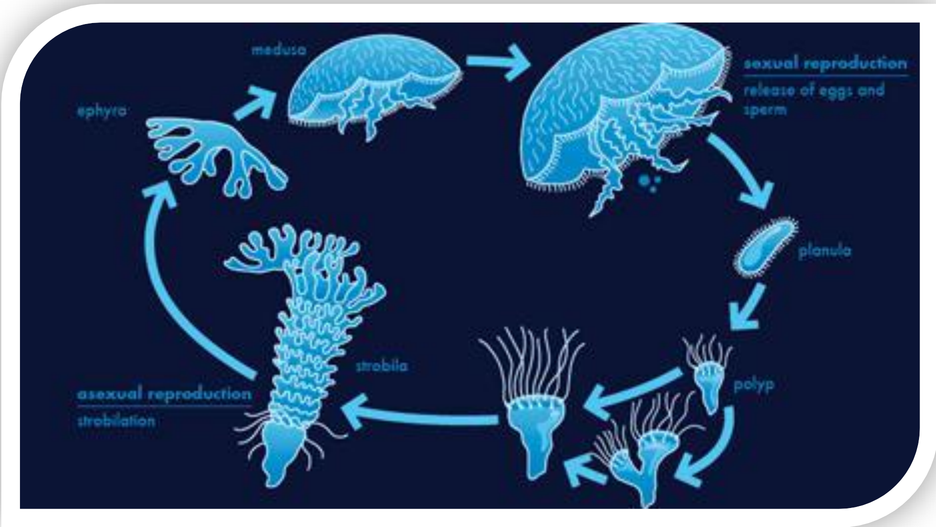


Figure – 2 Scyphozoa lifecycle depicting its 2 stages of sexual and asexual reproduction.

Adapted from Widmer (2008)

The medusa under study in the present MsC thesis, the *Chrysaora quinquecirrha* is carnivorous and its diet is composed of a wide variety of prey items although the most abundant usually are small crustaceans and other jellies (comb jellies and medusae included) (Purcell, 1992). Since medusae are able to capture a large amount of prey (true for quantity and larger prey size) their predation rates are on par with the quantity of food available meaning that even while undergoing digestion of a large quantity of prey they are still able to keep a high capture rate. For smaller medusae, diet choice experiments conducted in Chesapeake Bay showed a larger preference for copepods. Even though other prey were ingested like fish, larvae and other comb jellies, it is suggested that population pressure or control exerted by *Chrysaora* predation falls, exclusively, on local copepod populaces especially during summer seasons. (Purcell, 1992).

In natural conditions *Chrysaora quinquecirrha* and other medusae are known to capture a lot of bycatch prey that they are unable to ultimately digest. Common prey like bivalves specifically *Mytilus edulis* and *Mulinia lateralis* are caught through the oral arms but ultimately disposed of. (Purcell, 1991)

III. 3. Natural diet and Feeding behaviour

Most Scyphozoans possess oral arms around their mouth region, *chrysaora quinquecirrha* possess four, and these four arms usually hang from the underside, with many nematocysts present inside, used to inject potential prey and predator alike.

Oral arms of *Chrysaora quinquecirrha* contain many nematocysts, these organelles reside in subcellular regions of explosive cnidae cells. Nematocysts are highly venomous and are injected directly into prey/predators through the use of tubule shafts from the Cnidae cells. At the ephyra stage many scyphozoans including *chrysaora* possess nematocysts capable of paralyzing prey bigger than their size (very few are needed to paralyze even small arthropods). They are also utilized for taxonomic identification since many scyphozoans look very identical at the ephyra stage. (CALDER, 1977)

According to a series of experiments done on Chesapeake Bay regarding natural and laboratorial feeding efficiency of *Chrysaora quinquecirrha*, the presence or absence of prey medusae pulsation velocity and acceleration were altered as was area shift and concentration (Matanoski, 2001). When prey was absent, pulsation and acceleration shifts were much slower, relative depth was not related to prey availability but shifts between surface and depth were more heightened during feeding times. *Chrysaora* commonly uses swimming patterns to create high-velocity flow over the exumbrella margin generating currents that entrain zooplankton prey and delivers them to the tentacles and oral arm regions. (Matanoski, 2001).

In more recent years Schyphomedusae have been receiving added interest and close monitoring in order to better ascertain their role in the food web (Tanaka, 2001). Most diets will consist of preying on zooplankton; while these differ in situ they still complement a huge part of any medusa's respective food web. Seasonal movement and migration can also shift and incur specific changes to a medusa's diet (Gordon, 2012), environmental factors and temperature are known to influence dispersal and position along the water column, coastal and bay depth will naturally impact what changes will occur in feeding.

A small percentage of adult medusae diet can extend beyond copepods and other common zooplankton to complement other species of comb jellies (smaller double cell layered medusae), cnidarians and some small species of fish.

Out of the three prey items trituration of *Aurelia aurita* poured into ephyra tanks presented the overall biggest net growth over the 1 month trial period. It also presented the biggest growth for young ephyras (first day to 2-3 weeks) although after said period ephyras fed with copepods, had similar growth rates to the ones fed *Aurelia aurita*. Further conclusions about end result growth could only be drawn if the experiment had a longer duration than one month.

III. 4. Growth factors and Blooms

Seasonal blooms are a common occurrence among jellyfish in the wild. Many increases in magnitude and frequency have been linked to human alterations of coastal ecosystems as many subsequent blooms can have profound impacts on tourism, fisheries and commerce (Hays, 2011), (Collins, 2002).

Whether the frequencies of these events are of natural cause or anthropologically driven, any further understanding and identification of the separate factors is always invaluable considering the many potential risks of other populations when conditions are ideal for jellyfish blooms.

If said system is jellyfish dominated there is always concern regarding maintenance of other fish stocks, even more so, when coerced with local fishing burdens (Hays, 2011).

Plankton feeding phase is always essential for all fish, playing a crucial part in fish stock recruitment (Hays, 2011). Being unable to use sight as a direct tool for predation leads to an unfair advantage over other fish - the use of tactile predation provides additional feeding times during night where many visual based predators sleep or remain inactive

(Collins, 2002), (Matanoski, 2001). One of these recently studied aspects were different levels of dissolved oxygen and depth for the settlement of planulae and polyps of medusae and direct affiliation to growth/bloom occurrence. While depth and natural nutrient distribution affect oxygen dissolved in water, in many cases of coastal human activity the increase in natural waste provoking eutrophication tend to significantly drop D.O (dissolved oxygen) rates in water (Human development of the coastline leads to increased nutrient loading, resulting in decreased dissolved oxygen concentrations in coastal areas) (Hays, 2011).

While oxygen depletion does impact jellyfish growth negatively it seems to always have a larger effect on other species (namely larger fish). Studies indicate that hypoxia may benefit pelagic medusae, by decreasing zooplankton escape responses, thus, improving prey capture rates of medusa (Miller 2012). Fish and benthic invertebrates, or the potential predators and competitors of settling cnidarian planulae, are sensitive to low oxygen, making this environment suitable for most cnidarians (Miller 2012). Data indicates that tolerance to the physiological stresses of hypoxia in the scyphozoan polyp stage and the reduction of sessile competition and predation in hypoxic areas may make some of these areas particularly vulnerable to jellyfish blooms (Miller 2012).

III. 5. Jellyfish rearing and aquarium precautions

Jellyfish play in many ways an unknown part (outside bloom occurrences) in carbon and nutrient cycles as they themselves are usually comprised of 97% water and 3% organic matter. They are however capable of assimilating large percentages of protein from their prey and excreting phosphorous, nitrogen and carbon in large quantities (in relation to their organic mass) thus making it crucial for the renewal of aquarium water to be as assiduous as possible so as to not create build up (Frost, 2012). Although small in size the added waste of medusae and their prey, or their subsequent decomposition in tank conditions of 10 liters can rapidly assimilate various types of bacteria which will affect overall ephyra health. Studies show that certain natural assemblages of bacteria may decompose medusae including ones that benefit from indirect release of phosphates and other carbon matter. This was also monitored and seen in some early stages of ephyra death where decomposition could occur rapidly if water conditions weren't ideal, most of the times due to any leftover chemicals used to clean newly constructed aquariums or the many small variations in aeration.

The last particular factor is somewhat hard to control as newly released and young ephyras are quite small in size and physical trauma can easily occur through small uncontrolled variations. Other aspects like their daily removal for measurement and cleaning can also incur a small risk in regards to their overall physical integrity (tentacles in ephyra are easily detached). Any handling aspect of ephyras must be very delicate until they've reached juvenile stage, which will inevitably be longer than the current experiment time frame. Although medusae tend to concentrate their swimming area based on prey and light, smaller ephyras subject to constant aeration will have a naturally harder time controlling locomotion, however pulsation and speed variations are more common when in the presence of heavily lighted regions or when abundance of food is available (Matanoski 2001). Not much is known of feeding efficiency of *Chrysaora quinquecirrha*, even less outside of Chesapeake Bay (USA) or lab conditions, but contact with prey has been shown to affect swimming behaviour of schyphomedusae in a few studies with *Aurelia aurita* and *Chrysaora quinquecirrha* where these swim faster and concentrate on first point of contact with prey (Matanoski 2001).

III. 6. The species under study: *Chrysaora quinquecirrha*

The species itself is very common along the Atlantic and Indo-Pacific; it usually displays a pinkish, brownish coloration. It contains radial symmetry and a gastrovascular cavity used for digestion. The adult *Chrysaora* jellyfish can attach most of its ctenophore prey using its umbrella, oral arms and tentacles while small ephyras are only capable of capturing and attaching much smaller prey or parts of them through means of their oral arms (Larson, 1986). Eventually any prey attached to the medusae is moved to the oral arms where it is transferred to the stomach through ciliary muscle movement (ciliary creeping, are the same muscle contractions used for locomotion). When prey size is large the arms help degrade/digest using protease based enzymes (digestion through peptide hydrolysis). This enables the digestion of several preys at the same time. (Larson, 1986)

Populations of medusae like *Chrysaora* are far from being in danger and are known to have very little natural predators; many, however, have potential useful pharmacological properties and uses, specifically in regards to biological toxin and chemical production/characteristics (Pane, 2013). As such, studies focused in their maximized growth potential through lab conditions/aquaculture carry massive potential, both in field studies of their ecological and physiological properties but also as a source of bioactive substances for the development of new drugs (Pane, 2013).

A particular investigation was conducted, which carried out the purpose of testing anti-oxidant and anti-tumor properties of nematocyst venom peptide from *Chrysaora quinquecirrha* (Balamurugan, 2009). Experiments were conducted where tumors were induced in mice through intraperitoneal injection of Ehrlich ascites carcinoma (EAC tumor model), and various doses of sea nettle nematocyst venom (SNV) peptide were administered in order to monitor the difference in survivability when compared to the standard drug 5-fluorouracil (20mg/kg). A medium dose of 4,2 ug/kg of sea nettle nematocyst venom gave the rats a similar survival time as the standard dose of 5-fluorouracil. (Balamurugan, 2009).

The results were comparable to that of the result obtained from the animals treated with the standard drug 5-fluoracil (20mg/kg bw) showing that SNV peptide possessed substantial antitumor activity.

Fluoracil is an anti-metabolite that inhibits synthesis of DNA and RNA specifically inhibiting thymidylate synthase as a means of DNA production adjusting process.

This enzyme is directly connected to regulatory functions of DNA and RNA strand building, absence or defects related to this enzyme usually result in genetic and severe growth abnormalities.

While 5-fluoracil is extremely effective it is common to only target higher concentration regions and has been known to have a variety of side effects (The Scott Hamilton CARES Initiative).

SNV peptide has been shown to be a more controlled natural alternative of antioxidant and antitumor source than both 5-fluoracil and early experiments with Naja species snake venom in the delay of progressing adenocarcinoma cells. With snake venom their high curative properties are hindered by their high level of toxicity unless the patient has developed some degree of immunity through natural or SI (self-immunization) means (Balamurugan 2009).

III. 7. Objectives

Aquaculture and optimum growth of medusae is crucial for various sea trade institutions, more so for the continued research on medical properties and uses for nematocyst venom of various jellyfish species.

One of the crucial factors for growth in aquaculture being what food is available and how it is made available to the animal that is being reared, as such any potential diet and feeding plan can be mixed and optimized. For this particular experiment three diets were trialled over the course of 31 days, each one pertaining to three different Kreisler replicate tanks. These diets were; *Artemia salina* nauplii fed with 18 hour

SELCO enrichment, *Acartia grani* copepods fed with *Rhodomonas*. Sp*.* and *Aurelia aurita* fed with a mixed copepod and *Artemia salina* diet.

As an important predator it has the ability to alter and exert control in population densities, for many coastal systems it is, however, very difficult to chart swimming or feeding behavior for ephyra as they are very small in size. This was tested in this thesis first hand for ephyras *Chrysaora quinquecirrha* in small tanks. However, it was accomplished only very superficially, through the monitoring of their respective pulses after food was placed (with the objective of creating a pulse model for the different replicates and diets). The objective being not the difference in pulses from the presence or absence of prey/food but the difference in pulse behaviour regarding what food item was available and it's relation to ephyra age and development, basic perception of said item and if pulsation could be relatable to current ephyra size.

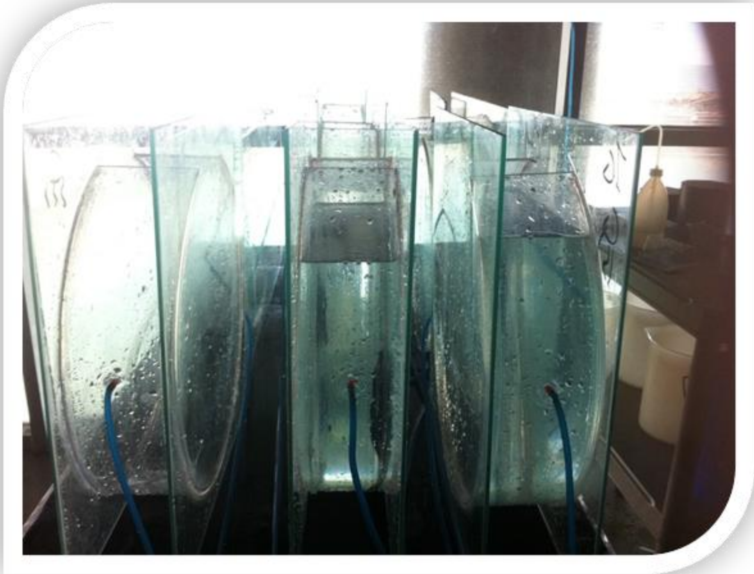
IV. METHODS

IV. 1. Ephyra tank construction

Aquarium construction was done with simple polystyrene plaques and glass panes, all materials were provided by project VITAL PTDC/MAR/111304/2009 coordinated by Dr. Susana Garrido: 18 glass panes approximately 80x70 cm (height, width), acrylic plastic cut out into 9 open end cylinders 12 cm in width and about 45 cm in height, silicone glue used to hold acrylic cylinder into the glass panes, drilling of one hole on the centre side of each acrylic cylinder and the attachment of one small tube for each aquarium (should be glued on to prevent detachment), central aeration system and enough tubes to build an aeration system for the 9 aquariums, bleach clean all 9 aquariums and remove excess glue letting them rest for about 1-2 days.

Figure -3 Front side view of ephyra aquariums inside the Lisbon Oceanarium

Figure -3 lateral view of ephyra aquariums inside the Lisbon Oceanarium



IV. 2. Experimental set-up

Chrysaora quinquecirrha polyps were collected from tanks in the Lisbon Aquarium Lisbon Oceanarium and temperatures were shifted so budding could occur.

Each set of 3 replicate tanks per diets could only start when at least 30 viable ephyras would be released. Viability would be achieved if ephyras would have at least 1.2 mm and no more than 1.5 mm in diameter with no physical abnormalities such as missing limbs or conjoined umbrellas.

Experiments consisted on providing 3 different diet compositions to ephyras of *Chrysaora quinquecirrha*, given at an approximate concentration of 500 individuals of *Artemia salina* and *Acartia grani* per litre of water, all except *Aurelia aurita* diet kreisler replicates which were fed a triturated solution of water and juvenile *Aurelia*.

10 ephyras were placed in each replicate tank, at three replicates for each diet a total of 90 ephyras were required.

48 hour *Artemia nauplii* have to receive a SELCO enrichment treatment of at least 18 hours, *Rhodomonas* sp. have to be maintained in tank cultures for the feeding of *Acartia grani*, *Aurelia Aurita* has to be carefully maintained and monitored in a tank with various individuals in different life stages.

IV. 3. Brief introduction towards food items utilized for experiment

- ***Artemia nauplii***

Also known as Brine shrimp it is the most widely used food source as it is convenient and easy to maintain. These small crustaceans are easily capable of forming dormant cysts and are readily available year around in coastal areas. Reawakening these cysts is a simple matter of saltwater hydration and right tank conditions; these can be very flexible in terms of salinity and temperature.

Lipidic and fatty acid content however is very low for these shrimp, it is common to use enrichment through feeding. Substances like SELCO (self-emulsifying lipid concentrate), super SELCO are very common as a means of augmenting HUFA content for *Artemia nauplii.*

SELCO is rich in highly unsaturated fatty acids containing particularly high levels of omega-3 fatty acids, Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA).

Usually 3 to 4 drops per litre of water is enough to enrich thousands of nauplii. It is not supposed to be viewed as a feeding alternative but rather a short lived emulsion to bolster dietetic value. *Artemia* are known to be very nonspecific feeders and a small amount of SELCO is enough to enrich a large population. Usually time immersed in SELCO treatment determines prey value for other aquacultures, recommended emulsion times usually round about 16 to 24 hours.

Of the three diets utilized, *Artemia salina* in its natural state is the one with the lowest nutritional value, even with the common use of SELCO as an enrichment source; they still have a great difficulty in retaining a higher nourishing value over copepods and other aquaculture foods.

They are larger in size than *Acartia grani* copepods, (the larger size makes digestion rate for ephyras slower) this diet adopts a regulator role or a helpful means of determining steady growth and comparison to the other potential diets, as growth rate is never expected to maximize under an *Artemia* exclusive diet.

- ***Acartia grani***

Acartia grani are calanoid copepods a class of zooplankton best described as free a swimming planktonic crustacean and one of the most abundant metazoans in the world. Capable of surviving in a very wide array of natural conditions these creatures are able to survive in a temperature range and shift of 30 Cº (-1 to 31 Cº) and salinities of 1 ppt to 38 ppt. Frequently found in coastal waters, estuaries lagoons and lakes. (Gonzalez, 2013)

These small creatures range from 0.5 mm to 1.5 mm with fairly translucent bodies (capable of observing ingested food such as *Rhodomonas* sp).

Fertilized eggs are very small and slowly sink; as such they are easily siphoned and transferrable to different tanks, when cleaning excess plankton and/or waste, it is important to separate them through the 3 stage of adult juvenile and eggs as the adults tend to predate on recently hatched juveniles. Maintaining salinity temperature and consistent lighting schedules is crucial to keep a healthy growing population.

They are fairly easy to rear females produce eggs for 3-4 weeks releasing clutches of about 20-53 eggs every 4 days (Gonzalez, 2013) although they do not achieve population densities anywhere near *Artemia salina* and have a slower growing rate females will survive around 70 days while males will survive less than half the amount of time, they contain a much higher nutrient value than even SELCO enriched *Artemia* nauplii. Ephyra growth results were quite positive under *Acartia* diet regime, initial growth was slow but towards the last week diet assimilation and growth became leveled with that of *Aurelia aurita*.

- ***Aurelia aurita***

Aurelia aurita is a translucent jellyfish with about 30 cm in diameter when reaching adulthood, found in most tropical and warm coasts they are able to adapt to large variations of temperature (-6 to 31 Cº). It is highly carnivorous and feeds on zooplankton, crustaceans, copepods and other small hydromedusae. While it holds no conservative status of note like many medusae it can exert major trophic changes and easily dominate small water systems during high temperature seasons. Out of the 3 prey items it is the hardest to maintain/grow, feeding used to maintain a healthy and thriving population takes a long effort both in resources and time, especially if the duration of the experiment will be over a certain time frame (if we are talking about the duration of several months and *Aurelia aurita* is cultured and not harvested locally, then It is safe to assume that to grow them you would need them several months in advance and a considerable amount of live copepods and crustaceans with which to feed them). Wild caught jellyfish usually present problems by carrying algae and bacteria from outside and contaminating a closed Kreisler tank and are more susceptible to suffer from temperature fluctuations.

IV. 4. Setting up aquariums

Trial periods for all tanks were 31 days starting on the strobilation day.

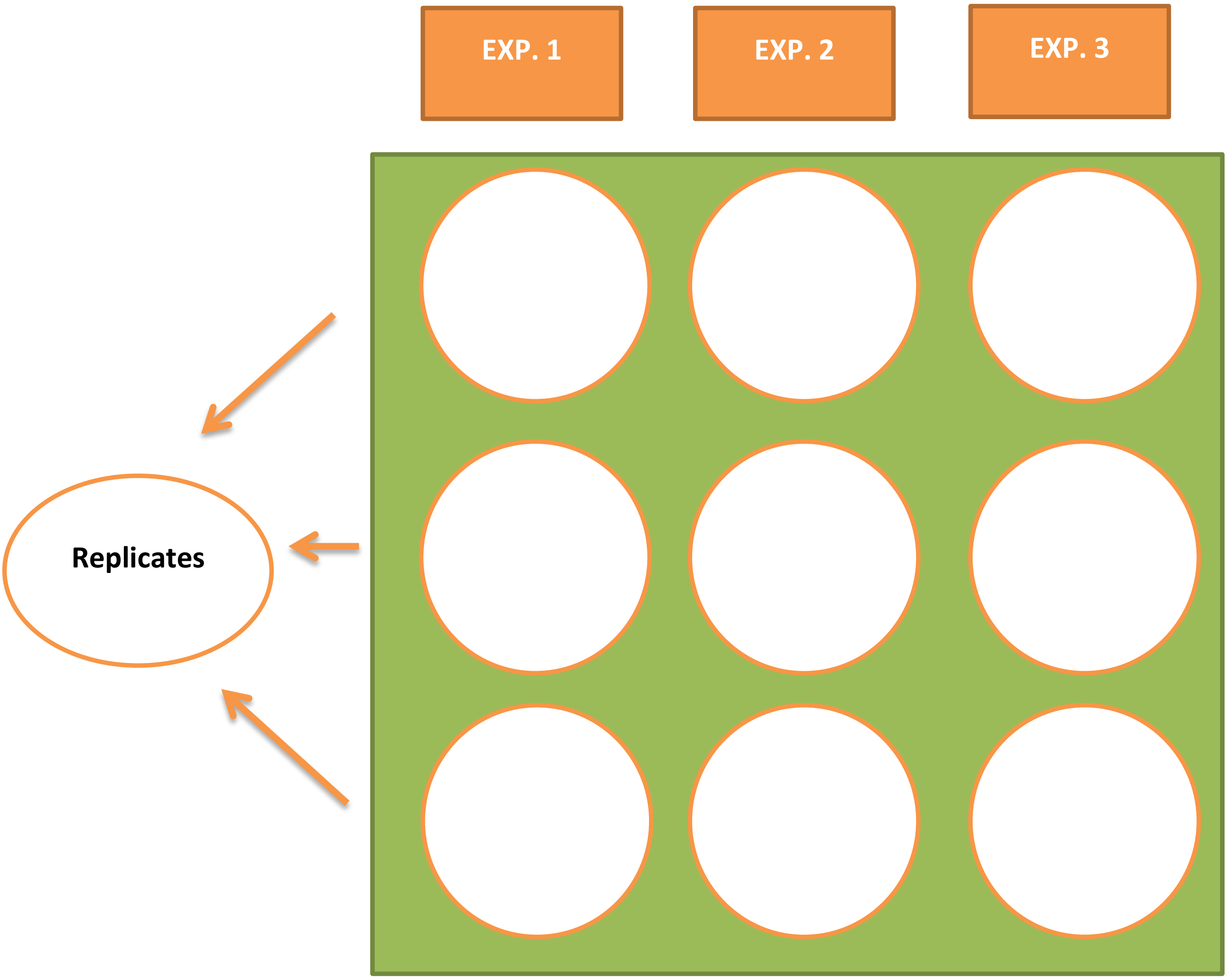


Figure – 4 Bird's eye view of experimental setup

- **EXP. 1:** ***Artemia salina*** nauplii enriched with **SELCO**
- **EXP. 2:** ***Acartia grani*** enriched with ***Rhodomonas salinam***
- **EXP. 3:** ***Aurelia aurita*** paste (minced ***Aurelia auritas*** fed with enriched brine shrimp nauplii)

- All recipients had an equal volume of 10 litres of brackish water with 60 percent inverse osmosis clean water and 40 percent UV filtered saltwater

- All recipients were cylindrical in shape with side aeration creating an anticlockwise current, keeping ephyras and prey in motion.

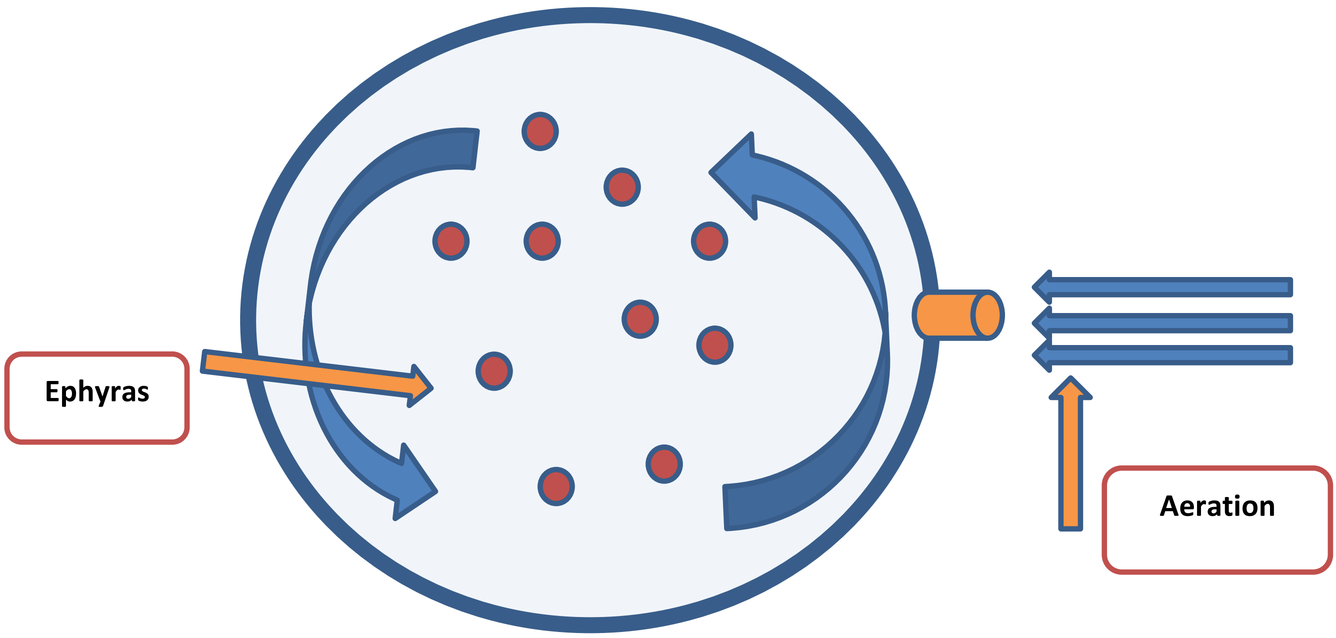


Figure – 5 *Airflow and current disposition inside the Kreisler replicates/tanks*

- The lighting regiment was 14h of artificial lighting followed by 10h darkness. The lighting was as homogenous as possible so as to not concentrate prey on lighted regions.

- Constant lateral aeration was maintained as variations are a common occurrence for small aquariums.

Ephyra measurements were taken on the first day and then once every other day, as described below.

IV. 5. Day to day routines

Tank cleaning and measurement procedures were as follows, collecting of ephyras from each tank with a pipette into separate replicate labeled containers, this was necessary to be able to perform a 100% water change for each tank daily. Careful cleaning and siphoning of *Artemia nauplii*, *Acartia grani* and *Aurelia aurita* presence inside the Kreisler is essential in order to minimize waste accumulation and maintain nutritional value of available food, (it is especially essential for *Artemia salina*, as SELCO supplementation loses effect after 24 hours very rapidly). *Artemia salina* and *Acartia grani* rearing tanks were changed and bleached once a week through method of rotating between available tanks. *Acartia grani* had 4 separate tanks with 3 active at any point of the experiment, and one that remained inactive until the bleaching process was over. 3 active tanks were separated as follows, one contained *Acartia* in its egg stage, the second contained *Acartia* in its juvenile stage and the third one would have *Acartia* in its adult form. Separation is necessary as adult *Acartia grani* prey on juvenile *Acartia*.

Measurement of 5 random ephyras per tank in diameter was done once every two days so measurements would have some small significant difference.

Measurement process was as follows:

1. Place ephyras in petri dish with only a few drops of water to restrict movement.

2. Measurement of diameter with a micrometre ruler.

Figure – 6 Representation of an ephyra

3. Refilling of aquariums with specified previous standards of brackish water (60% inverted osmosis clean water and 40% UV filtered saltwater)
4. distribution of food accordingly (500 individual *Artemia naplii* and *Acartia grani* per litre in respective tanks, and 1 adult or 2 juvenile *Aurelia aurita* distributed along the 3 respective replicates).

IV. 6. Statistical analyisis for ephyra growth

Histograms were utilized to verify data normality, after which a Levene test was utilized to verify homoscedasticity between variables of each set of diet replicates, in order to achieve this, further manipulation of data was required and so medusa diameter was log-transformed. With this all diets fulfill the homoscedasticity principle, all variances are significantly correlated to random sampling of invidual ephyras and all replicates for each respective diet have some significant difference in size value between replicates and so a covariance (ANCOVA TEST) and mixed model analysis is applied. An Ancova analysis is done to verify significant differences between replicates of each treatment (verifies if covariance between variables is linear ie. Has a trend/tendency or not). ANCOVA analysis assesses mean values of diameter in relation to age with the covariance factor being each replicate for each specific diet. Since replicates don't have the same value for slopes and intercepts in unison between them we have to use a mixed model analysis so that the resulting linear equation takes into account that the replicates are not all the same, since the element of covariance was found to be significant for each replicate in individual diets. This was followed with a mixed effects analysis in order to measure random effect variance between replicates for each of the diets. A mixed affects analysis is conducted in order to measure random effect variance between replicates for each of the diets. Meaning, with a simple linear equation can we define mathematically in what way does diet influence ephyra growth without introducing the replicate variable into the equation. This variable was accounted as a random effect within the mixed model and was used to determine what impact other conditions besides food had on ephyra growth Results.

IV. 7. Statistical analyisis for ephyra Pulses

Histograms were used to test data for normality. All histograms show a significant degree of normality in Pulse values, although the values themselves seem to produce no significant pattern or correlation to age or food given. To further test this, a Levene test was used once again to analyse homoscedasticity in variables. In the end ANCOVA models were made to verify differences in pulse frequency between replicates for each of the diets.

V. GROWTH RESULTS

V.1 Growth rate for replicates of each diet

V.1.1.1 - Replicates with an *Artemia salina* diet

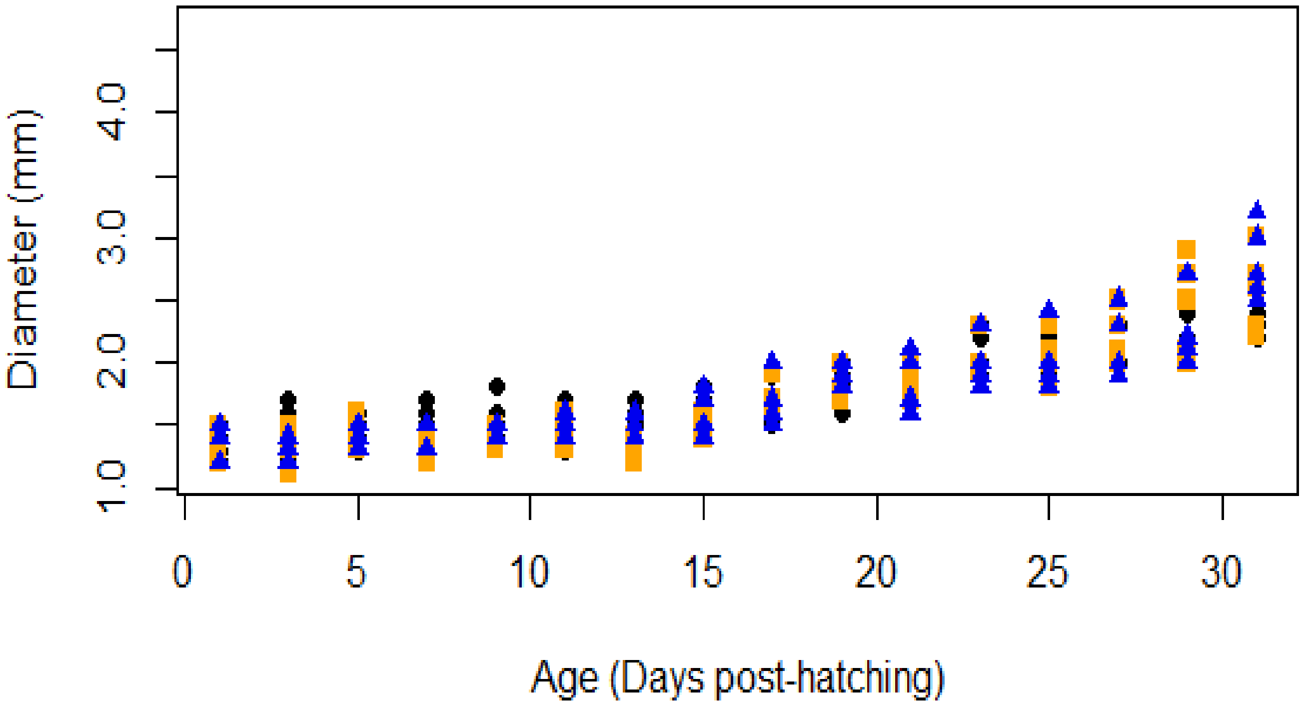


Figure – 7 Total Diameter length (mm) for the 3 replicates of *Artemia salina over the course of the 31 day experiment.* ***Replicate 1(A1), Replicate 2(A2), Replicate 3(A3).***

Mean ephyra size at the day of strobilation was 1.38mm ± SD, 1,32mm ± SD and 1.3mm ± SD respectively (A1, A2, A3) for the replicate tanks fed with *Artemia salina*. The third replicate had the highest mean size of ephyra (2.8mm ± SD) by the end of the 31 day trial; out of the 3 diets *Artemia* had the lowest final diameter or total growth). The intercept value for day 1 of the 3 replicates was not significantly different

A(I) = 1.282 however growth slopes for the 3 replicates was significantly different. A1(s)=0.030, A2(s)=0.019, A3(s)=0.0205 respectively.

V.1.1.2 – Histogram for *Artemia salina* growth

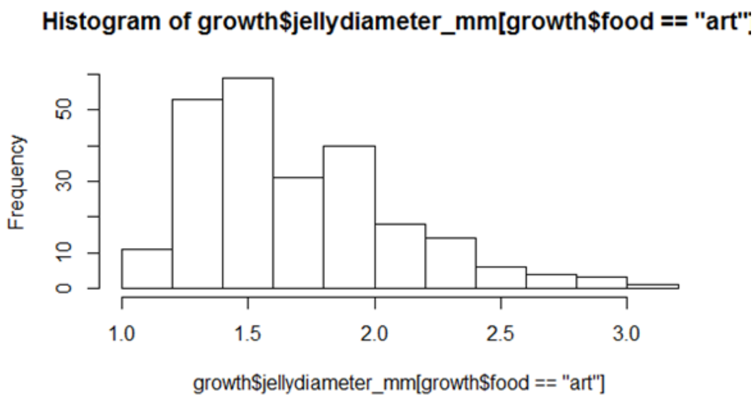


Figure – 8 Histogram depicting data distribution for ephyra growth (mm). Ephyra diameter followed a reasonably normal distribution along the *Artemia salina* diet as visually inspected in the histogram. Further analysis with a Levene test showed a P value of (P=0.1664) for the size variable within the 3 replicates of this diet. Since value exceeds 0.005 the homoscedasticity principle is maintained.

V.1.1.3 – ANCOVA TEST for *Artemia salina* Diet growth

For Artemia all 3 slopes(s) were different in value for size, however intercepts(i) were identical with a value of 1.282:

A(s)=0.030

B(s)=0.019

C(s)=0.0205

V.1.2.1 - Growth results for Replicates with an *Acartia grani* diet

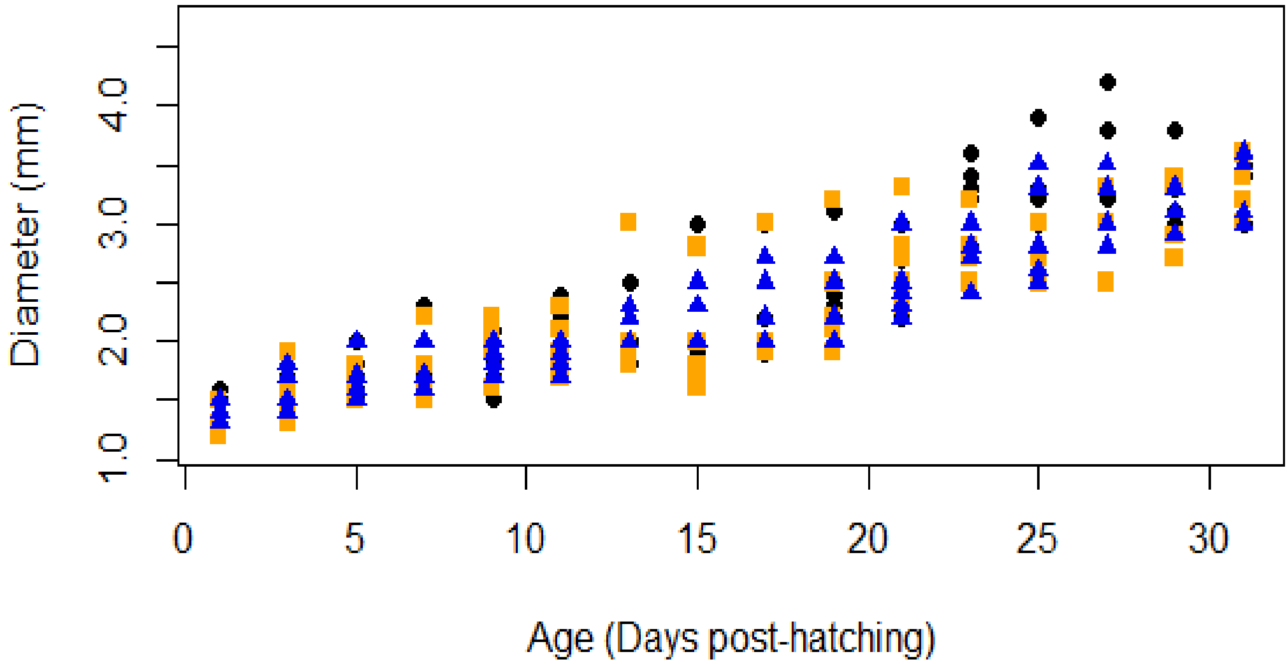


Figure - 9 Total Diameter length (mm) for the 3 replicates of *Acartia grani* over the course of the 31 day experiment. ***Replicate 1(B1), Replicate 2(B2), Replicate 3(B3).***

(All replicates for *Acartia grani* started with mean values of 1.4mm ± SD, 1,3mm ± SD and 1.38mm ± SD respectively (B1, B2, B3). The first replicate had the highest mean value of 3.52mm at day 27, since sampling is done with 5 individuals at random it is expected that mean values will have fluctuations towards the final quarter of the experiment. Some individuals will have a significant difference in size. Largest size in *Acartia* diet was recorded for an individual ephyra reaching 4.2mm in diameter. The intercept value for day 1 of the 3 replicates was significantly different B1(I) = 1.362, B2(I) = 1.2237, B3(I) = 1.2499 however growth slopes for the 3 replicates was not significantly different B(s) = 0.066.

V.1.2.2 – Histogram For *Acartia grani* growth

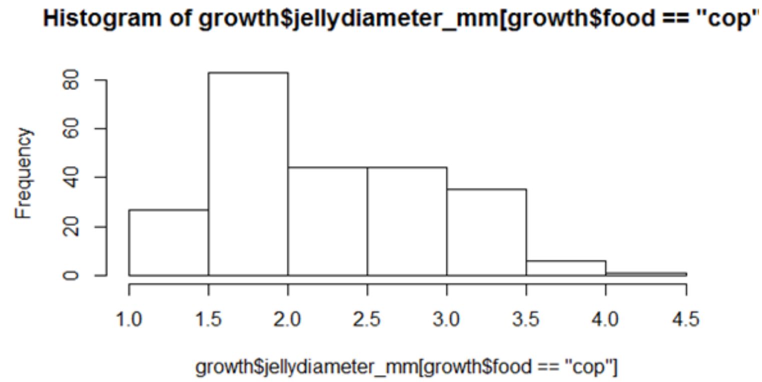


Figure –10 Histogram depicting data distribution for ephyra growth (mm). Ephyra diameter followed a reasonably normal distribution along the *Acartia grani* diet as visually inspected in the histogram. Further analysis with a Levene test showed a P value of (P=0.1192) for the size variable within the 3 replicates of this diet. Since value exceeds 0.005 the homoscedasticity principle is maintained.

V.1.2.3 – ANCOVA TEST for *Acartia grani*Diet growth

For *Acartia grani* slopes(s) had identical values in regards to starting size (mm) of 0.066, but intercepts(i) were different:

A(i)=1.3620

B(i)=1.2237

C(i)=1.2499

V.1.3.1 - Growth results for Replicates with an *Aurelia aurita* diet

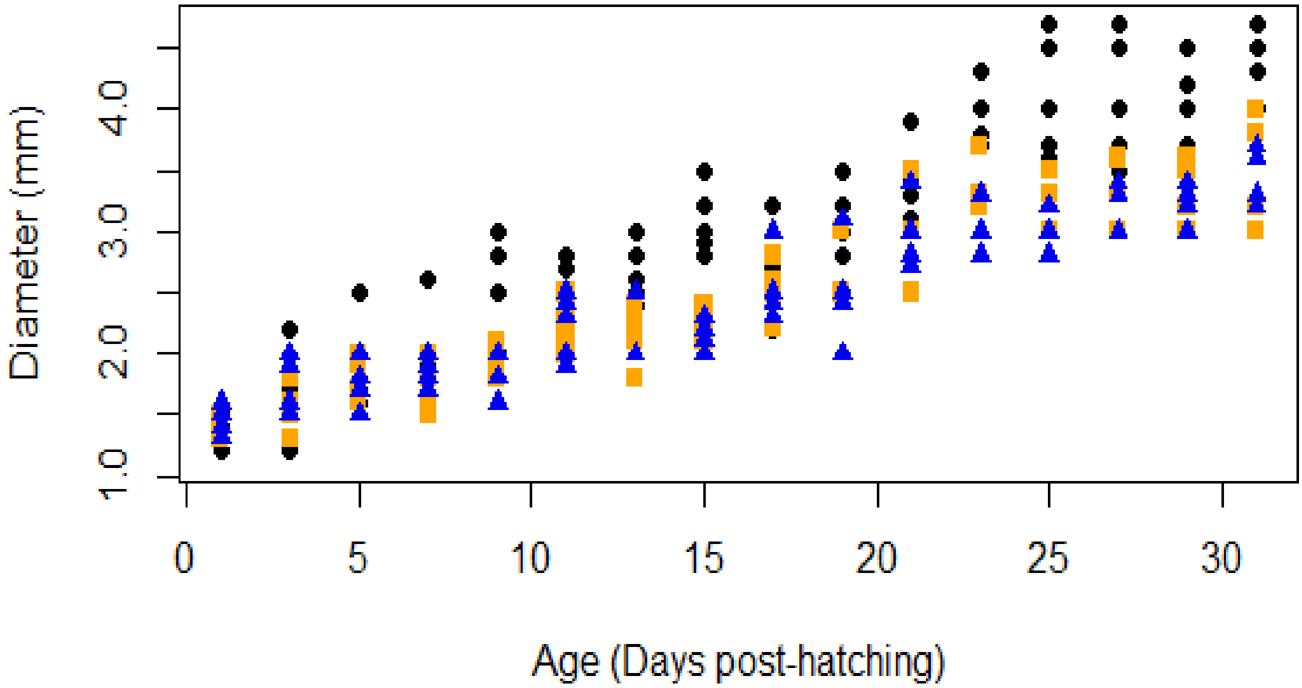


Figure - 11 Total Diameter length (mm) for the 3 replicates of *Aurelia aurita* over the course of the 31 day experiment. ***Replicate 1(C1), Replicate 2(C2), Replicate 3(C3).***

All replicates for *Aurelia aurita* started with mean values of 1.34mm, 1,4mm and 1.42mm respectively (C1, C2, C3). The first replicate had the highest mean value of 4.3mm at day 31. Of the 3 diets *Aurelia aurita* recorded the largest growth in ephyra diameter). The intercept value for day 1 of the 3 replicates was not significantly different C(I) = 1.388 however growth slopes for the 3 replicates was significantly different. C1(s)=0.0975, C2(s)=0.0744, C3(s)=0.0664 respectively.

V.1.3.2 – Histogram for *Aurelia aurita* growth

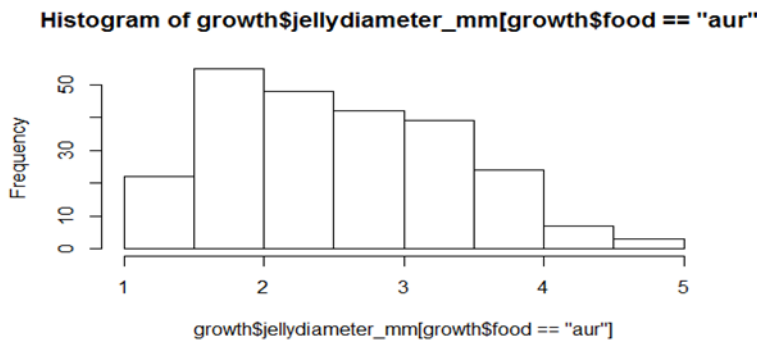


Figure – 12 Histogram depicting data distributions for ephyra growth (mm). Ephyra diameter followed a reasonably normal distribution along the *Acartia grani* diet as visually inspected in the histogram. Further analysis with a Levene test showed a P value of (P=0.0005958) for the size variable within the 3 replicates of this diet. In this case since P value is lower than 0.005 in order for the homoscedasticity principle to be applied, size values have to be logged to change size variability between them, in order to be able to apply further parametric testing.

V.1.3.3 – ANCOVA TEST for *Aurelia aurita* Diet growth

For *Aurelia aurita*, intercepts had identical values of 1.388, but slopes(s) were different:

A(s)=0.0975

B(s)=0.0744

C(s)=0.0664

V.2 Comparing ephyra growth in different diets

When comparing the growth rates of ephyras fed with different diets through a mixed model using diet as a factorial variable and replicate as a random effect, results show that the growth of ephyra fed minced *Aurelia* was significantly higher than growth rates provided by copepods and these were significantly higher than those fed with *Artemia salina.*

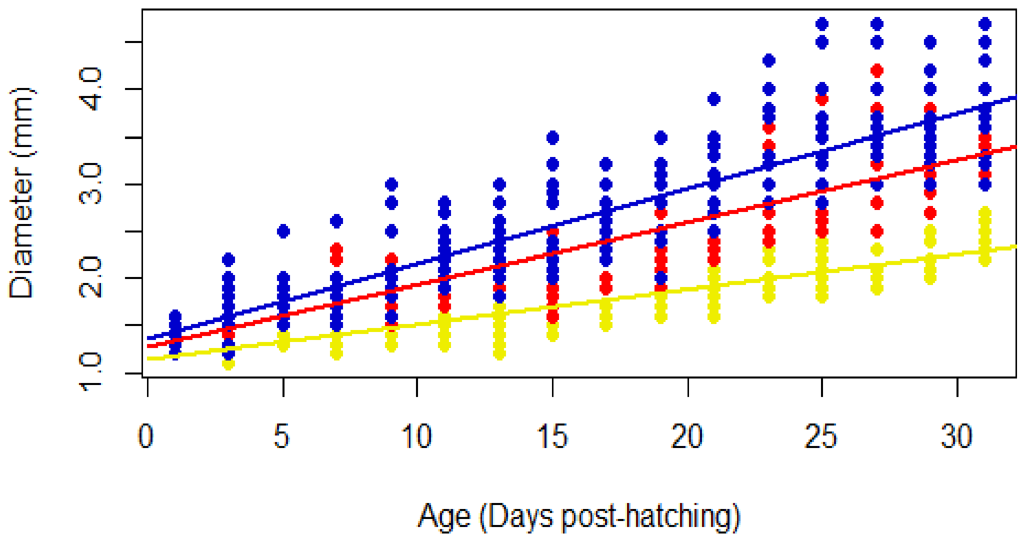


Figure 13 – Growth rates were; ***Artemia salina: Size ~ 1.149 + 0.037age***, ***Acartia grani: Size ~ 1.278 + 0.0657age***, ***Aurelia aurita: Size ~ 1.361 + 0.07946age.***

V.3 Pulse results and Statistical analysis

V.3.1.1 - Pulse results for *Artemia salina* replicates

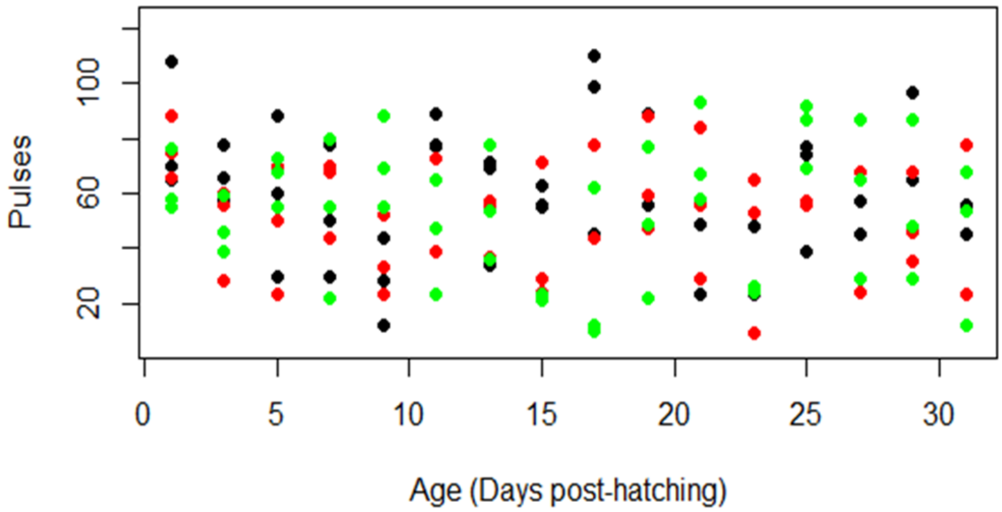


Figure - 14 Pulse variations for the *Artemia salina* replicates moved between 11 and 110 (Pulses per minute ±SD) and no significant correlation was found between ephyra size and pulse frequency ***Replicate 1(A1), Replicate 2(A2), Replicate 3(A3).***

V.3.1.2 - Histogram For *Artemia salin*a Pulses

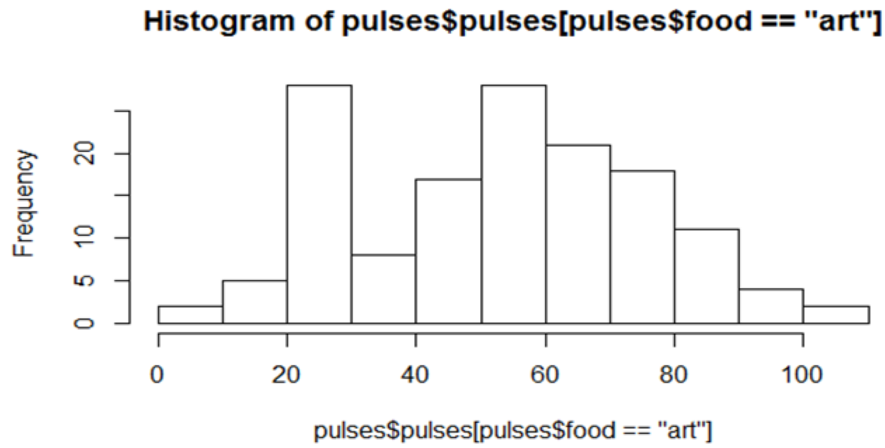


Figure – 15 Histogram depicting data distribution for ephyra pulses (number of pulse per minute). Ephyra pulse frequency followed a reasonably normal distribution along the *Artemia salina* replicates as visually inspected in the histogram. Further analysis with a Levene test showed a P value of (P=0.8362) for the pulse frequency variable within the 3 replicates of this diet. Since value exceeds 0.005 the homoscedasticity principle is maintained.

V.3.2.1 - Pulse results for *Acartia grani* replicates

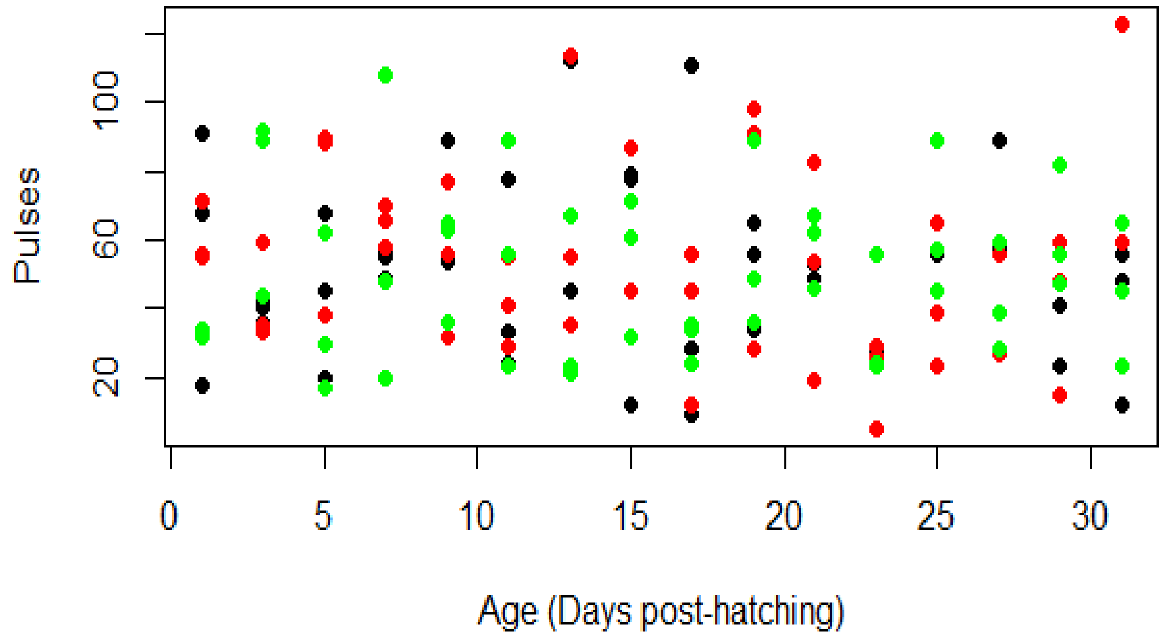


Figure - 16 Pulse variations for the *Acartia grani* replicates moved between 5 and 123 (Pulses per minute ±SD) and no significant correlation was found between ephyra size and pulse frequency ***Replicate 1(B1), Replicate 2(B2), Replicate 3(B3).***

V.3.2.2 - Histogram For Acartia grani Pulses

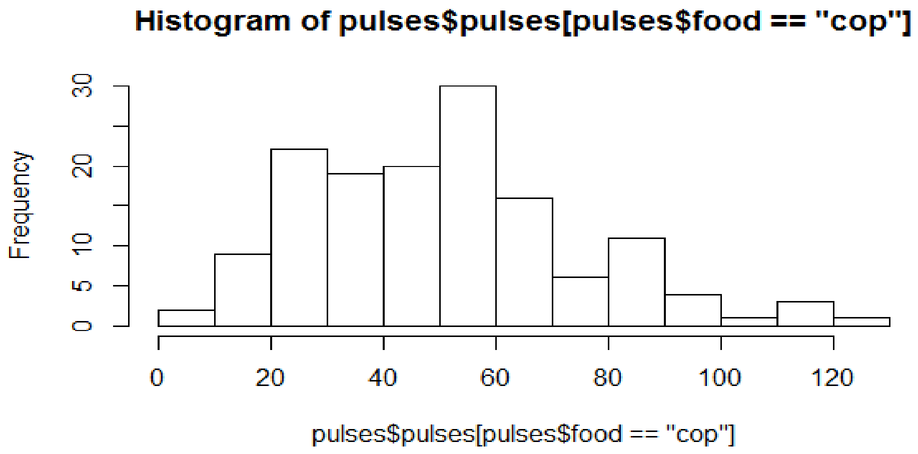


Figure – 17 Histogram depicting data distribution for ephyra pulses (number of pulse per minute). Ephyra pulse frequency followed a reasonably normal distribution along the *Acartia grani* replicates as visually inspected in the histogram. Further analysis with a Levene test showed a P value of (P= 0.8649) for the pulse frequency variable within the 3 replicates of this diet. Since value exceeds 0.005 the homoscedasticity principle is maintained.

V.3.3.1 - Pulse results for *Aurelia aurita* replicates

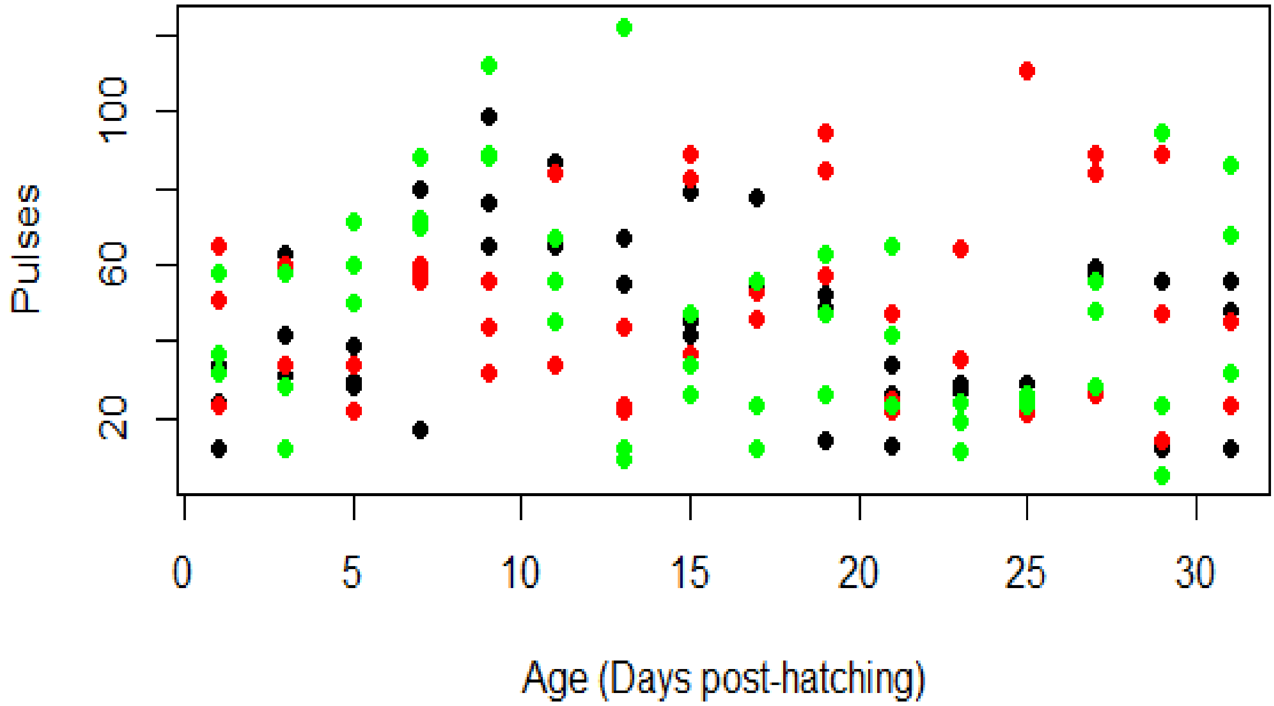


Figure - 18 Pulse variations for the *Aurelia aurita* replicates moved between 5 and 122 (Pulses per minute ±SD) and no significant correlation was found between ephyra size and pulse frequency ***Replicate 1(C1), Replicate 2(C2), Replicate 3(C3).***

V.3.3.2 – Histogram For *Aurelia aurita* Pulses

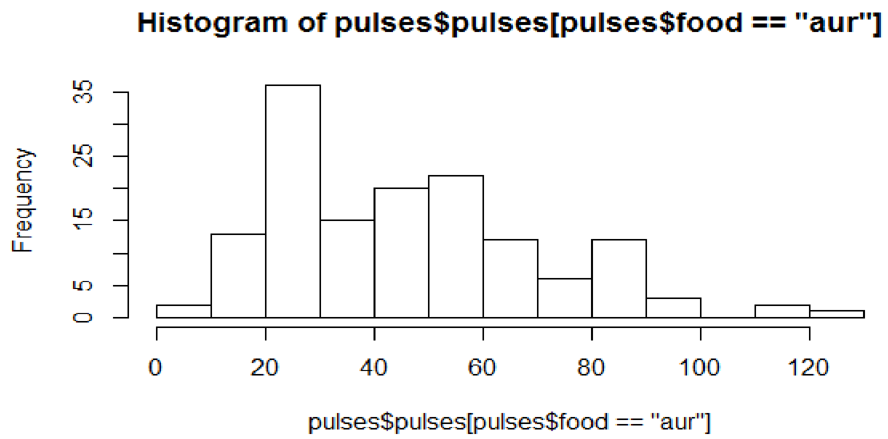


Figure – 19 Histogram depicting data distribution for ephyra pulses (number of pulse per minute). Ephyra pulse frequency followed a reasonably normal distribution along the *Aurelia aurita* replicates as visually inspected in the histogram. Further analysis with a Levene test showed a P value of (P= 0.2924) for the pulse frequency variable within the 3 replicates of this diet. Since value exceeds 0.005 the homoscedasticity principle is maintained.

V.3.4 – Statistical analysis for Ephyra Pulses

All histograms show a significant degree of normality in Pulse values, although the values themselves seem to produce no significant pattern or correlation to age or food given.

The frequency of pulses was not significantly different between replicate tanks therefore there was no need to statistically analyse the difference of pulses between the diets using linear mixed models, and an ANOVA model was applied.

The ANOVA model comparing the pulses between medusa fed different diets revealed no significant differences between them. When all data were pooled to study the influence of ephyra age in the frequency of pulses, it was observed that pulses slightly decreased with age, following the equation:

$$\text{Pulses} = 54.98 - 0.277 \cdot \text{Age}.$$

VI. DISCUSSION (DETAILS)

VI. 1. Summary

The primary aim of this study was what food items were superior towards the aim of ephyra growth under specific aquaculture conditions. Since all tanks were identical in material and construction, other factors like, numbers of individuals per tank and food quantity were also matching; aeration was visually mediated between tanks but 100% certainty is hard to ascertain. Any major difference would have to be attributed to starting weight/size of ephyras and respective tank diet. Subsequently differential behaviour is hard to discern and was only monitored through means of individual pulsation of a number of individuals per tank over time, most results proved to be inconclusive as any statistical correlation between pulse values and age or diet were not significant.

In the department of marine sciences in the University of Puerto Rico *Chrysaora* growth was tested with a ctenophore and medusae exclusive diet of *Mnemiopsis leidyi, Mnemiopsis mccraydi* and *Cassiopeia frondosa* on juvenile *Chrysaora* ranging in weight from 25 to 300 grams, where net growth varied in 2% for larger specimens and 10% for the smaller specimens (Larson, 1986). Ctenophores commonly known as comb jellies are very similar to the medusae but have 2 cell depth internal and external layers and are a very common natural prey item for *Chrysaora* especially in the western part of the Atlantic Ocean.

VI. 2. What the key differences in diets were?

Artemia salina

Artemia is one of the most commonly used aquarium diets it's use harbours more of a means of control of maintaining a given population than actual effective growth the low HUFA content present in *Artemia* (Marini, 2012) even with the help of SELCO supplementation it has proven to be very ineffective in medusae growth. The largest tank mean value for ephyra diameter was 2.8 mm by the end of the experiment, which is very low when compared to the other diets. In initial growth patterns the growth rate was staggering slow only significantly changing by the end of the second week. That being said it is however the diet in regards to self-upkeep with the lowest time and economic input, requiring little maintenance and proving to be relatively quick to rear, if SELCO is added it is important to note that feeding times must be carefully monitored in order to maximize effect (Marini, 2012).

Acartia grani

Copepodes make up a large sum of an ephyras natural diet even with older medusae since fatty acid content is high and their relatively small size allows capture and digestion rate to be maximized (Gonzalez, 2013). In most ephyras observed it was quite common to catch them mid digestion with two or more copepods (the rate was significantly higher than with *Artemia*, while for *Aurelia aurita* it is visually impossible to observe the digestion process). The highest mean value in size ranged from 3.24 to 3.52mm approximately, due to large variations in size between individuals, these variations could be attributed to behaviour, aquarium currents, ephyra structural integrity and initial sizes as some had a 0.1-0.3mm variation between them.

In regards to growth, copepods prove to be the most cost effective diet, while significantly superior to *Artemia* in nutritional value; they are harder to rear and still demonstrated lower mean growth values for ephyras than the *Aurelia aurita* diet. Their growth curve however, significantly increased by the end of the third week, almost matching that of *Aurelia aurita*. Through means of a mixed effects analysis it was shown that mathematically both *Acartia* and *Aurelia* curves seem to be much more approximate in value towards the end of the experiment.

Aurelia aurita

The jellyfish's diet consists mostly of plankton (Larson 1986), but in their natural surroundings they also eat a variety of other things including: fish eggs, larvae of other marine animals, molluscs crustaceans, and will sometimes eat other jellyfish (Tanaka, 2001).

However, phenomenon such as intra-specific cannibalism tends to be very rare. A jellyfish's tentacles are lined with stinging cells, these nematocytes fire off microscopic quills that lodge in a victim and pump in venom (their tentacles are not inherently poisononous). However, jellyfish have chemoreceptors that can turn nematocytes on and off (Burnett, 1998), and these chemoreceptors can recognize the chemical signature of its own species (Gibbons, 2010).

Inter species cannibalism is common between jellyfishes albeit being much more common in adult sized medusae (Tanaka, 2001) than in small sized ephyras, nonetheless trituration in a blender has proven to be an effective method of administering *Aurelia aurita* as a food resource to juvenile ephyras, the biggest drawback being when comparing quantities with the other diets.

For the 3 diets *Aurelia aurita* was the one that had the largest ephyra growth rate with the highest mean values ranging from 4.3 to 3.56 mm, like in the copepod diet certain individuals differed in size quite extensively.

The rearing *Aurelia auritas* is a very time consuming process, the ones utilized for feeding were almost adult in size taking several months to reach specified dimensions. Additionally, extensive resources and aquariums are needed to maintain and feed them, making it a risky endeavour with much previous preparation. Having enough or surplus moon jellyfish for the trial experiment is essential, and any shortage or depletion mid experiment will basically terminate it for a long period of time.

VI. 3. What key questions were answered?

1. Rearing of jellyfish from an ephyra stage is quite challenging, growth is still relatively slow during first month but still key differences in diet are quite evident at this stage.

2. Even though it is not a frequent part of an ephyra's natural regime, a diet of *Aurelia aurita* proved to be the most effective and probably would maintain this status if the experiment were longer.

3. It is possible to have conclusive results with a low individual count (10 per tank) while not common practice, results were still quite satisfactory with the usage of 3 replicates per diet.

4. Out of three diets the optimum diet for aqua culturing would be the use of copepods *Acartia grani* as they yielded very effective results and are relatively easy to maintain/grow.

5. If the only concern is fast growth of a given population then recommendation would be the usage of *Aurelia* aurita.

6. Tactile predation is crucial towards influence of feeding times, light disposal is also essential during experiments, not as a direct visual aide, during hours where tanks were absent of light this was not a complete impairment itself in feeding activity - food was only changed daily, maintaining optimum concentrations of prey *Acartia grani, Aurelia aurita* and *Artemia nauplii.*

VI. 4. Personal Recommendations

1. *Artemia* are very similar in size to ephyras, removing ephyras out of the tanks to measure proved to be a very time consuming process, special care and attention is required and advised when treating tanks with *Artemia salina* and new-born ephyras.

2. Tanks must be properly cleansed and checked for structural flaws, some initial problems occurred where some tanks had small incisions and others had not been

given enough time for the bleach to dissipate. Resulting in some incidents where experiments had to be restarted midweek.

3. The tanks are fitted with small aeration tubes due to their shape and size, these are prone to small variations in intensity over time, the consequence this could have was not directly measured or analysed, as such long term effects are hard to summarise. However in some cases oscillations between very low and very high intensities might have resulted in some isolated ephyra deaths.

4. Time scheduling is very important, if initial planulae were more abundant, then all tanks could've started at the same day, there would've been enough individual ephyras to start all three diets and the experiment could've been concluded earlier.

5. With more individuals per tank instead of just 10 per replicate, size sampling could've been more extensive, possibly eliminating some spiked mean size variations recorded along the final weeks.

6. With more individuals per tank you also eliminate substantially the risk of restarting a tank's diet due to lack of viable sampling population, (ephyra deaths are relatively common during the first week).

7. Ephyra structure should be carefully viewed microscopically early on to ascertain integrity all around so as to not have it influence movement and feeding later on in the experiment.

8. Careful extraction of ephyras is paramount as they can easily die under small durations of physical trauma. On several occasions a few tanks were reset due to significant loss of individuals.

VI. 5. What future research may be done?

1. Tanks could be bigger in size and capacity with individual peripheral aeration for each one, instead of a centralized unit, as this will greatly reduce oscillations in tank aeration flow.

2. If in future longer trial experiments are conducted they will provide an invaluable hands-on approach to quantifiying food along growth gradient and what diets would be more/less effective over *Chrysaora quinquecirrha's* life cycle.

3. Discerning effective measures for the discovering of the main characteristics and factors affecting ephyra pulsation?

4. If a longer life cycle is monitored will pulsation variation be attributed almost exclusively to age/size?

5. Was food quantity optimum? What would be the best method to ascertain this in parallel with ephyra growth?

6. What direct effects does lighting play in ephyra behavior/feeding?

7. What other diets can prove to be effective, given the high nutritional value of copepods and economical vantages, other fairly easy reared copepod species may prove to be effective, such as *Euterpina acutifrons* and *Tisbe* sp?

8. Can the right aquaculture conditions provide a faster rearing environment than natural habitat?

9. How would growth be affected in near anoxic conditions?

VI. 6. What questions remain to be answered?

The Atlantic sea nettle and other medusae are not known for having many natural predators (Graeme C. Hays, 2011) or becoming endangered by human management/activities or habitat shifts and anoxic conditions (Mary-Elizabeth C. Miller a, 2012). As such it is hard to justify aquaculture production or development of species that easily thrive and present problems to some local populations (J.C. Matanoski, 2001). Nevertheless they are an invaluable resource in the aquarium trade and further research will continue to help assert their practicality in the pharmacological field. In regards to oncological treatment investigation it is still very much in its initial stages but further use of nematocyst venom and further analysis of its properties will most likely become a fruitful endeavour for latter generations.

For future approaches to sea nettle diet, other species of copepods and other diets should be trialled since copepods seem to be the safest choice economically, the problem here being it’s potential effectiveness over a larger trial period or more adult stages of *Chrysaora quinquecirrha.*

Covering a wider array of stages in a medusa’s life cycle will help discern what diets work in what stages and if any can have a positive effect throughout the whole cycle.

If a more extensive lifecycle is covered perhaps pulsation can give a better insight into feeding ephyra behavior.

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